

Isolation and identification of prevalent bacterial pathogens from an exotic fish *Tilapia zillii* and *Oreochromis mossambicus*

N. Akila*, R. Sathi Kumaran

Department of Zoology, Pachaiyappa's College, Chennai, Tamil Nadu, India

Abstract

Aim: Fishes are susceptible to a wide variety of bacterial pathogens which are part of the natural microflora of the environment. The aim of the present study was to isolate the pathogenic bacteria in gills, liver, gut, and skin of *Tilapia zillii* and *Oreochromis mossambicus* from the lake near Perumbakkam in Chennai. **Materials and Methods:** The experimental fish *T. zillii* and *O. mossambicus* were collected from large lake 2 km length which starts from Perumbakkam through Vengaivasal Chithalapakkam in Chennai. **Results and Discussion:** Based on the differentiation and characterization of isolates on specific culture media, human bacterial pathogen such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Shigella dysenteriae* was isolated. In this study, the total plate count in the tissues of *T. zillii* ranged between 7.14×10^5 and 23.2×10^5 cfu/g. Similarly, in *O. mossambicus*, the total cell count in the tissue ranged between 6.21×10^5 and 21.16×10^5 , respectively. The isolation of enteric bacteria in fish serves as indicator organisms of fecal contamination and/or water pollution. Their presences are also hazardous to humans. **Conclusion:** The association of pathogenic bacteria among the fishes suggests that if fish are handled or prepared improperly it could lead to food safety hazards for consumers.

Key words: Bacteria, contamination, identification, *Oreochromis mossambicus*, *Tilapia zillii*

INTRODUCTION

Fish are good food to man and its flesh is rich in proteins, minerals, and fat. It is man's most important source of high-quality protein, providing approximately 16% of the animal protein consumed by the world's population.^[1] However, the fishes are susceptible to a wide variety of bacterial pathogens, which are capable of causing disease and are considered by some to be saprophytic in nature.^[2] The microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it.^[3] It has been suggested that the type of microorganisms that are found associated with particular fish depends on its habitat.^[4] The bacterial pathogens associated with fish have been classified as indigenous and non-indigenous.^[5] A great number of the coliforms are neutral to human, but their presence indicates that microorganisms are harmful to human. Bacterial species that are facultative pathogenic for both fish and human beings and may be isolated from fish without

apparent symptoms of the disease. Human infections caused by pathogens transmitted from fish are quite common and depends on the season, patient's contact with fish and related environment, dietary habits, and the immune system of the individual. However, some bacteria such as *Staphylococcus* spp. may be found only in the body surface of the fishes. The microbial count in different fishes has showed that the number of organisms was more on the body surface of big fishes with higher body weight than on the surface of small fishes because the organisms use the surface area of the fish as a microhabitat in their ecosystem.

Aquaculture products, from tropical regions, may harbor pathogenic bacteria which form a part of natural microflora

Address for correspondence:

N. Akila, Department of Zoology, Pachaiyappa's College, Chennai – 600 0030, Tamil Nadu, India.
E-mail: akila2004@gmail.com

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of fish ponds.^[4] Thus, water and fish quality are very important subjects to protect public health with a special emphasis on the microbiology. Human pathogens have been reported to be associated with different types of fish. The fish that live in water polluted with human and the animal fecal matter may carry considerable numbers of bacteria such as *Salmonella*, *V. cholera*, *E. coli*, and other coliforms. Human infections and intoxications caused by pathogens transmitted from fish are quite common and the following bacteria have been recorded: *Salmonella* spp., *E. coli*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Staphylococcus aureus*, and *Listeria monocytogenes*.^[6] Outbreaks usually occur due to the consumption of raw or insufficiently heat-treated fish, which may be contaminated with bacteria from water environment (*Vibrio* spp.) or terrestrial sources *Salmonella* spp., *Staphylococcus* spp., and *V. cholerae*.^[6] In the case of poor hygiene, the contamination of fish and fish products may increase due to unsanitary procedures, the rotation of the assigned duties of workers. Bacteria enter into the fish with food and water and accumulate in the intestine. Some of them retain their for a relatively longer period, but most of them can stay very temporarily due to incompatible environment produced from physical and chemical conditions, lethal interactions between bacteria and immune responses of the gut. The freshwater or rivers and lakes have a complex flora of microorganisms which include aquatic species as well as component introduced from terrestrial, animal, and plant sources.^[7] The human activity has a detrimental effect on coastal waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and will be concentrated by the filter-feeding activities of shellfish.^[7] While handling the fishes, it may be contaminated with organisms associated with man such as members of the *Enterobacteriaceae* and *S. aureus* which can grow well at 30–37°C.^[8] Various studies have suggested that intestinal microflora was responsible for various food spoilage^[9] and contamination of fish due to enteric bacteria of human or animal origin.^[10] The aquatic bacterial flora plays a significant role in the productivity of water, and there are also some potent organisms responsible for fish diseases. Identification of the microorganisms in various tissues of the fish would be of great value in correct interpretation of nutritional requirements of fish. Foodborne infection in relation to public health has been considered with great importance in the whole world. By monitoring the bacteria contents of fish organs, the quality of fish can be measured as they affect the storage life and quality of the fishery products.^[9] To provide a predictive capability for possible outbreaks and provide an opportunity to design preventive management actions, detailed information of the bacterial load and types of bacteria associated with the organs of healthy fish is needed. Considering these factors, the present study was undertaken to isolate and quantify the human pathogenic bacterial microflora in various tissues such as gill, liver, gut, and skin of *Tilapia zillii* and *Oreochromis mossambicus* that was caught from the pond.

MATERIALS AND METHODS

Collection of Sample

The experimental fish *T. zillii* and *O. mossambicus* were collected from large lake 2 km length which starts from Perumbakkam through Vengaivasal Chithalapakkam in Chennai. The fishes were collected aseptically and immediately transported in a thermal bag to the laboratory and processed within 3 h of acquisition, and samples were kept in the refrigerator (4–8°C).

Preparation of Stock Culture

Each organism was observed in the laboratory by macroscopic external for the identification of lesions in skin. A ventral section was then made to observe internal organs such as gills, liver, and gut. 1 g of the fish sample was dissected out, blended, and mixed properly in a mortar. It was aseptically transferred to a sample bottle containing 9 mls of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 min and allowed to stand for 20 min, after which a 10-fold serial dilution was carried out in duplicates, and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37°C for 48 h as described by Slaby *et al.*^[11]

Enumeration, Isolation, and Identification of Bacteria

Coliform organisms and Gram-negative enteric bacteria counts were determined using pour plate method with MacConkey agar and EMB agar, respectively. Mueller-Hinton agar for *Pseudomonas* spp., *Salmonella* spp., and *Shigella* spp. was enumerated using Salmonella Shigella Agar and thiosulfate-citrate-bile salt sucrose agar for pathogenic *Vibrio* spp. The plates were incubated at 35 ± 2°C for 24 h after which the colonies that developed on the plates were counted. Those counts within 30–300 colony-forming units (CFU) were reported as total viable count. Distinct colonies from each plate were then picked by means of a sterile wire loop and subcultured onto a freshly prepared nutrient agar medium contained in sterile plates. This was done with a view to obtaining pure culture of the growth. The plates were incubated at 37°C for 24 h.

Characterization of the pure isolates was performed and involved colonial characteristics, cell micromorphology, biochemical test of Gram reaction, catalase test, indole test, oxidase test, lysine test, methyl red test, and spore staining. These tests were done to identify isolates to generic level as contained.^[12]

Estimate of Mean (CFU/ml)

The mean CFU/ml denoted by \bar{x} was calculated as $\Sigma fx / \Sigma f$, where Σfx is the sum of the products of number of colonies

and the CFU/ml; while Σf is the summation of the number of colonies.

RESULTS

In this study, the total plate count in the tissues of *T. zillii* ranged between 7.14×10^5 and 23.2×10^5 cfu/g. The skin had the highest number of bacteria with 23.2×10^5 cfu/g in *T. zillii* [Figure 1]. Similarly, in *O. mossambicus*, the total cell count in the tissue ranged between 6.21×10^5 and 21.16×10^5 , respectively. The liver had the lowest isolation 7.14×10^5 cfu/g in *T. zillii* and 6.21×10^5 cfu/g *O. mossambicus* [Figure 2]. The *E. coli* was in the highest count 10.57×10^5 cfu/g in the gut of *O. mossambicus*, and it was low 5.93×10^5 cfu/g in the gill of the *T. zillii*. The liver and the skin were also populated with *E. coli*. All the tissues in both the fishes were highly populated with *P. aeruginosa*, the skin showing highest count of 35.12×10^5 cfu/g in *O. mossambicus* and 32.68×10^5 cfu/g in *T. zillii*, respectively. The *Shigella spp.* had a low isolation rate in the gill and gut of *O. mossambicus* with 5.10 and 5.26. *Enterococcus faecalis* had a low isolation rate in all the samples compared with other isolated organisms except *Shigella spp.* that had the lowest counts. Likewise, *Salmonella typhi* exhibited low isolation rate of 0.86×10^5 cfu/g in the skin of *T. zillii*. Table 1 indicated that the characterization used to identify the bacterial species isolated.

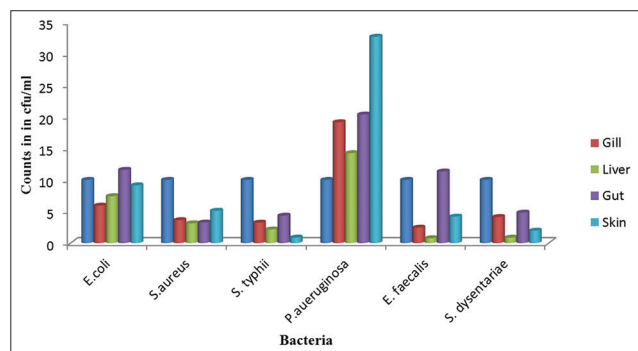


Figure 1: Total counts of the bacteria in colony-forming units/ml present in different parts of the fish

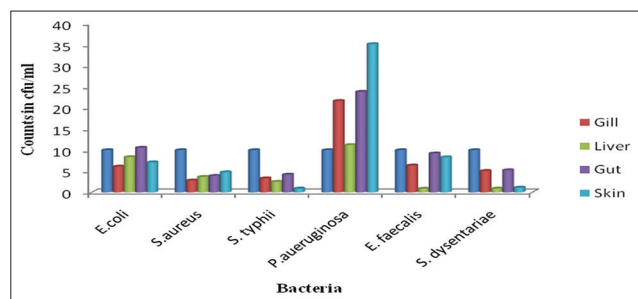


Figure 2: Total counts of the bacteria in colony-forming units/ml present in different parts of the fish *Oreochromis mossambicus*

The human bacterial pathogens that were isolated and identified include *E. coli*, *S. aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. faecalis*, and *Shigella dysenteriae*, as indicated in the table.

DISCUSSION

The presence of harmful bacterial flora was attributed to the contamination of the lake by animal waste.^[13] The isolation of *Salmonella*, *Shigella*, and *E. coli* from the fish samples indicates fecal contamination of the lake resulting from the livestock manure that they add to the fish ponds as feed. The isolation of *Salmonella*, *Shigella*, and *E. coli* indicates the environmental pollution.^[14] Coliforms such as *E. coli* are usually present where there is fecal contamination from warm-blooded animals.^[15] The organism *E. coli* is recognized as the reliable indicator of fecal contamination in small numbers and large numbers as it is an indicator of mishandling.^[16] *E. coli* is the only species in the coliform group that is found in the human intestinal tract and the other warm-blooded animals as a commensally and is subsequently excreted in large quantities in feces. Microbiological quality of the fish examined is unacceptable and it has a potential risk to public health. The diversity of potential pathogens from the samples of fish is of concern, particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. These opportunistic and pathogenic bacteria were also previously isolated by several other researchers from fish.^[17] The fishes in this study harbored human disease causing organisms that cause diseases such as food poisoning, diarrhea, typhoid fever, and Shigellosis. When these microorganisms such as *S. aureus*, *Salmonella*, *Shigella*, and *Pseudomonas* are present in the food, they are most likely to cause foodborne diseases.

The reports by Al-Harbi and Uddin^[18] indicate that the presence of bacteria in fishes digestive flora is normal. However, the outbreak of disease is related to the existence of a stress factor based on the interaction between fish, pathogens, and aquatic environment as a natural habitat of the organism, as well as poor water quality or excess of organic matter factors which allows the incidence of disease to be greater, as mentioned by Huicab-Pech *et al.*^[19] Although fish exhibit high bacterial diversity,^[20] points out that there is a symbiotic effect among bacteria, that is, the host adapts to nutritional changes and food assimilation in the digestive tract through bacterial balance.

In similar studies, *Escherichia coli*, *Pseudomonas aeruginosa*, *S. dysenteriae*, *S. aureus*, and *S. typhi* were isolated from the gills, intestines, muscle, and skin of *Megalaspis cordyla* and muscles of *Priacanthus hamrur* from Royapuram waters in India.^[21] This was attributed to the heavy load of sewage disposal into the seas which could act as a suitable environment for the growth and survival of the human pathogens. Similarly, the large lake was selected for the present study may also be

Table 1: Characterization used to identify the bacterial species isolated

Bacterial isolates	Colonial characteristics	Cell micromorphology
<i>E. coli</i>	Bright pink colonies	Appears as rod-shaped bacterium, Gram-negative
<i>S. aureus</i>	Golden yellow colonies appearing as grape-like clusters	Gram-positive, coccus shaped
<i>S. typhi</i>	Smooth pale colonies	Gram-negative bacilli
<i>P. aeruginosa</i>	Large, opaque, irregular colonies with fruity or earthy smell	Gram-negative rod shaped
<i>E. faecalis</i>	Colonies are circular, white, smooth, and shiny	Gram-positive cocci
<i>S. dysenteriae</i>	Circular, convex, colorless, colonies with smooth surface	Gram-negative bacilli

E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *S. typhi*: *Salmonella typhi*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *E. faecalis*: *Enterococcus faecalis*, *S. dysenteriae*: *Shigella dysenteriae*

polluted with sewage disposal in the neighborhood which would activate the growth and survival of human pathogens. Stringent regulations and monitoring activities coupled with food safety training of suppliers (fishermen and traders) and ultimately the consumers on various aspects of good hygiene practice, good manufacturing practice, and HACCP are strongly recommended.

CONCLUSION

The diseases are caused mainly due to several factors that act in an aquaculture; therefore, it is considered necessary to monitor the pathogens associated with fishes. The presence of highly pathogenic agents such as *Salmonella*, *Shigella* species, and of opportunistic pathogens is a potential health risk/hazard to human beings and may cause diseases to susceptible individuals, especially the immune-compromised consumers. The presence of potentially pathogenic organisms to humans in the fish suggests that if they are improperly handled, undercooked, or consumed raw they may contribute to the spread of the pathogens in the community. This research has brought to light those bacterial species associated with *T. zillii* and *O. mossambicus* and has shown that they are potentially pathogenic to humans. Hence, adequate measures should be taken in the examination of fish, especially for the presence of pathogens, during handling, storage, and up to the very point of consumption is needed for the protection and maintenance of community health by keeping foodborne diseases to a minimum.

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